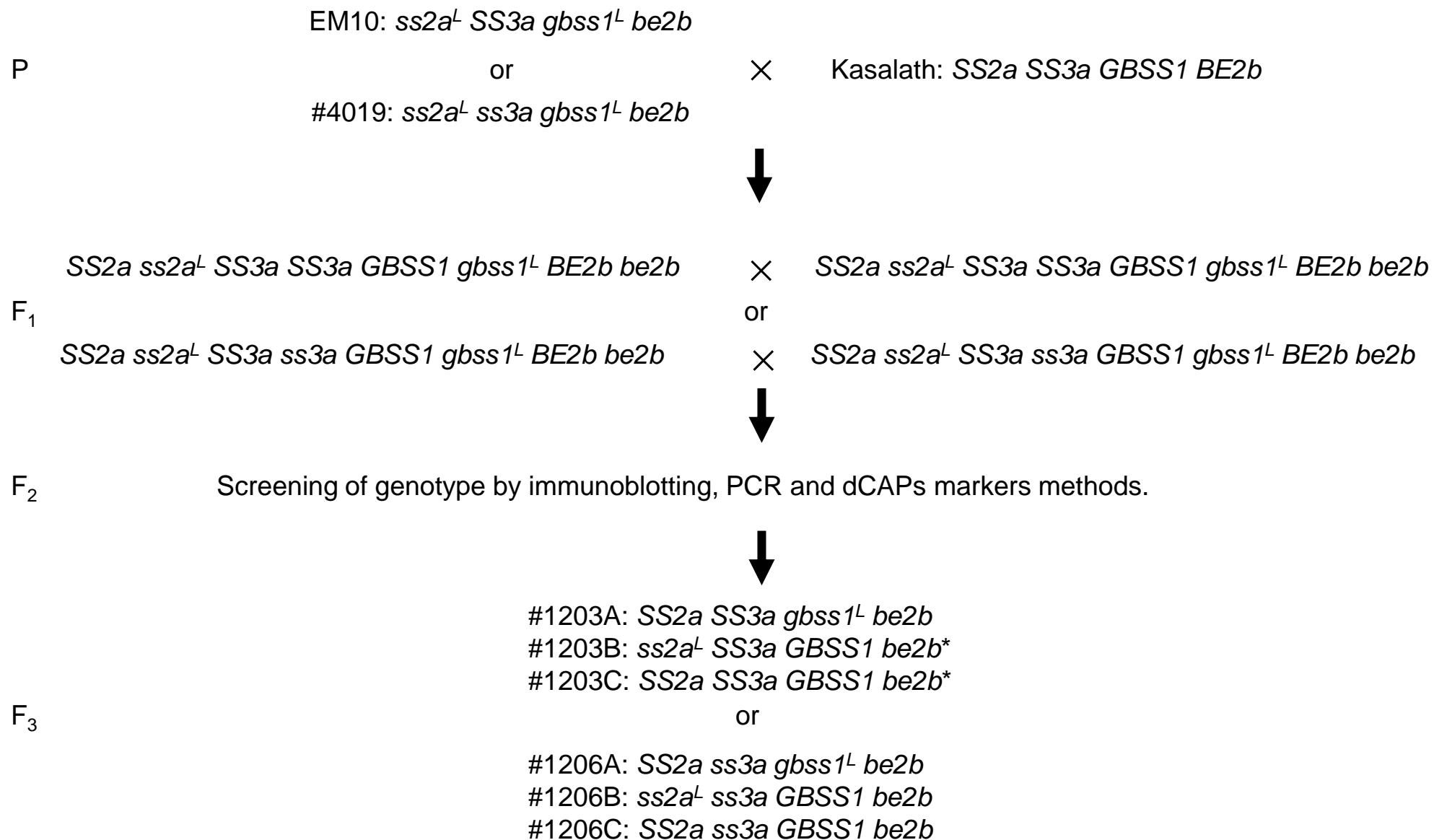
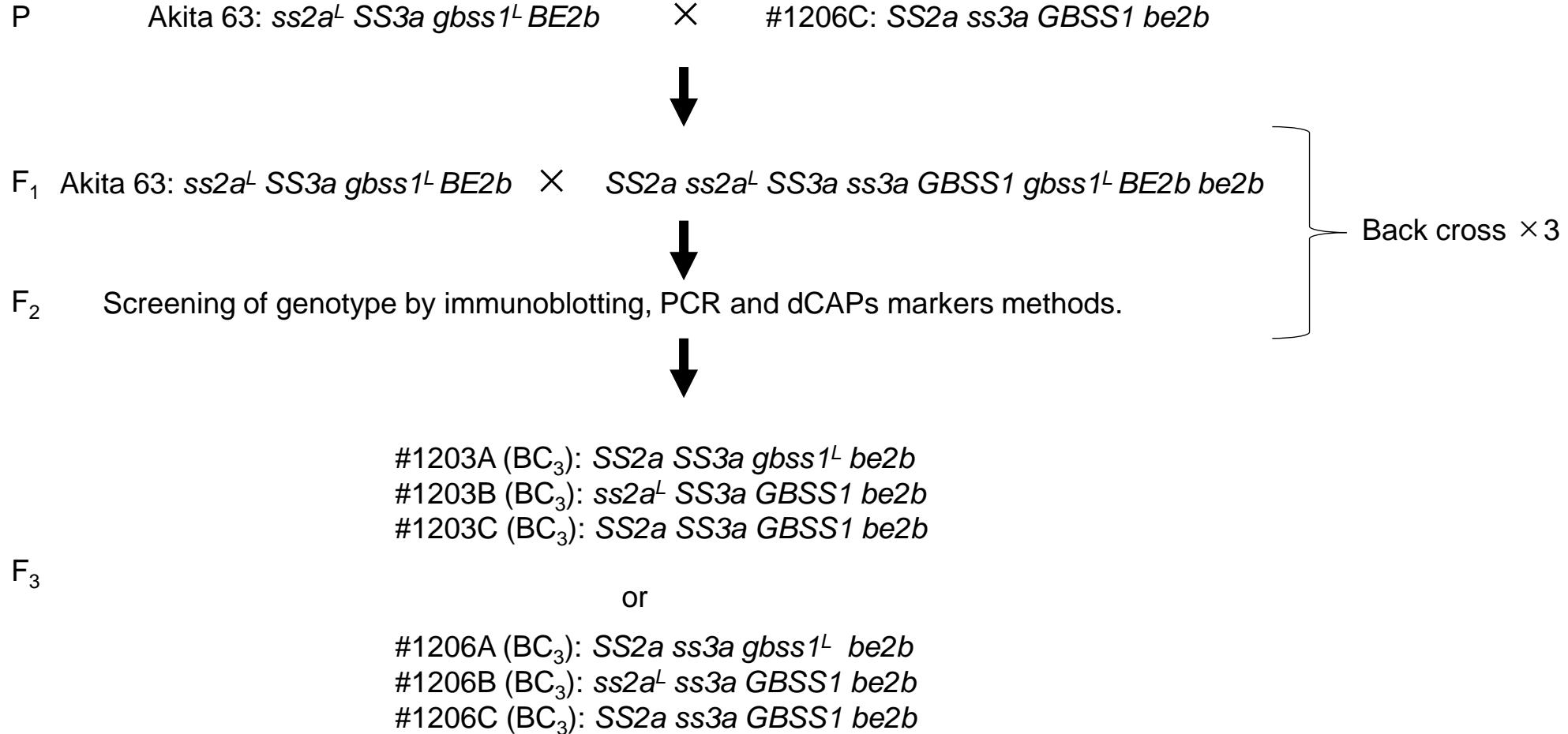


(A) Before backcrossing

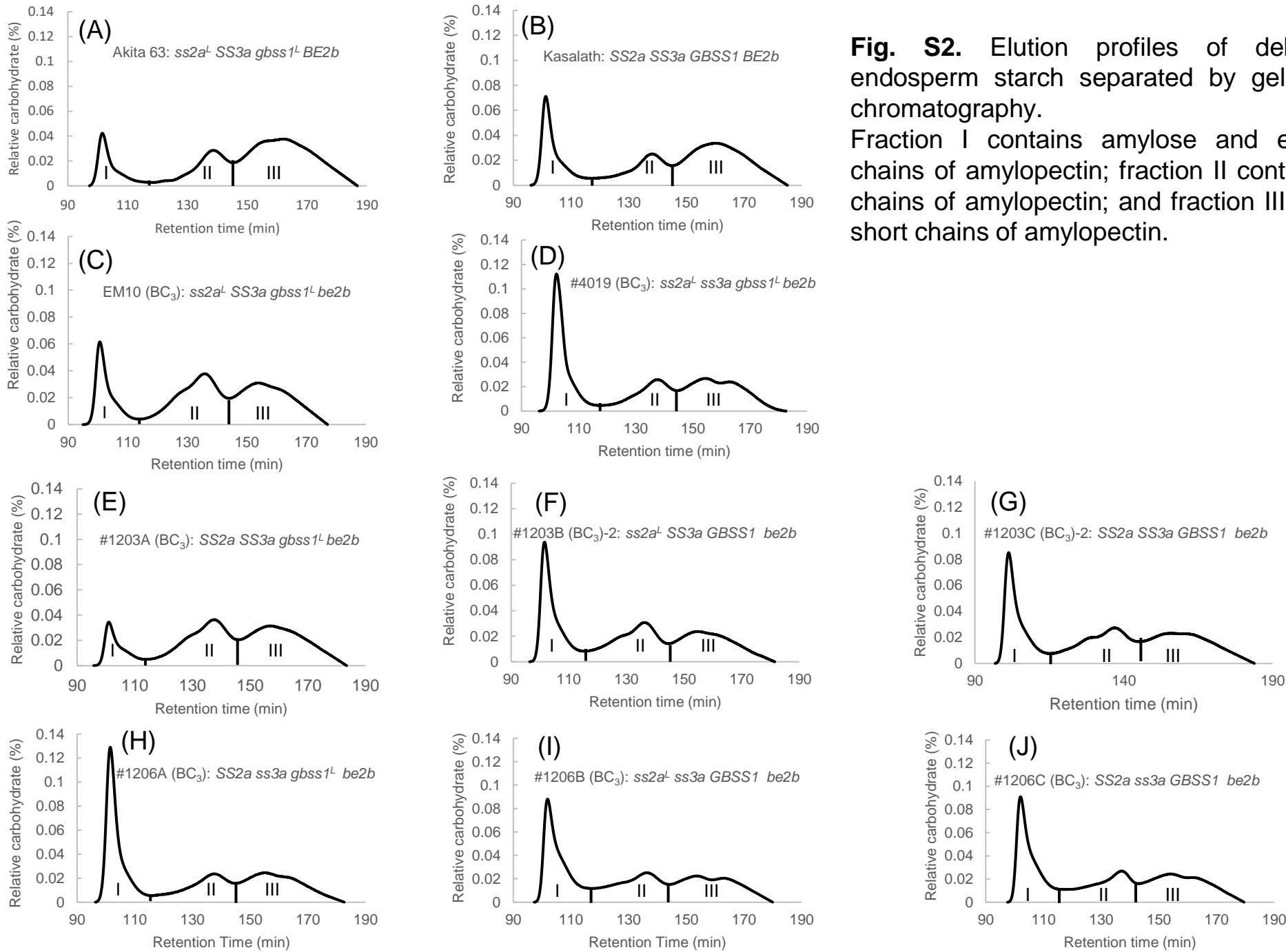


**Fig. S1A.** Pedigree of #1203 and #1206 lines before backcrossing. \*The lines previously described (Itoh et al. 2017).

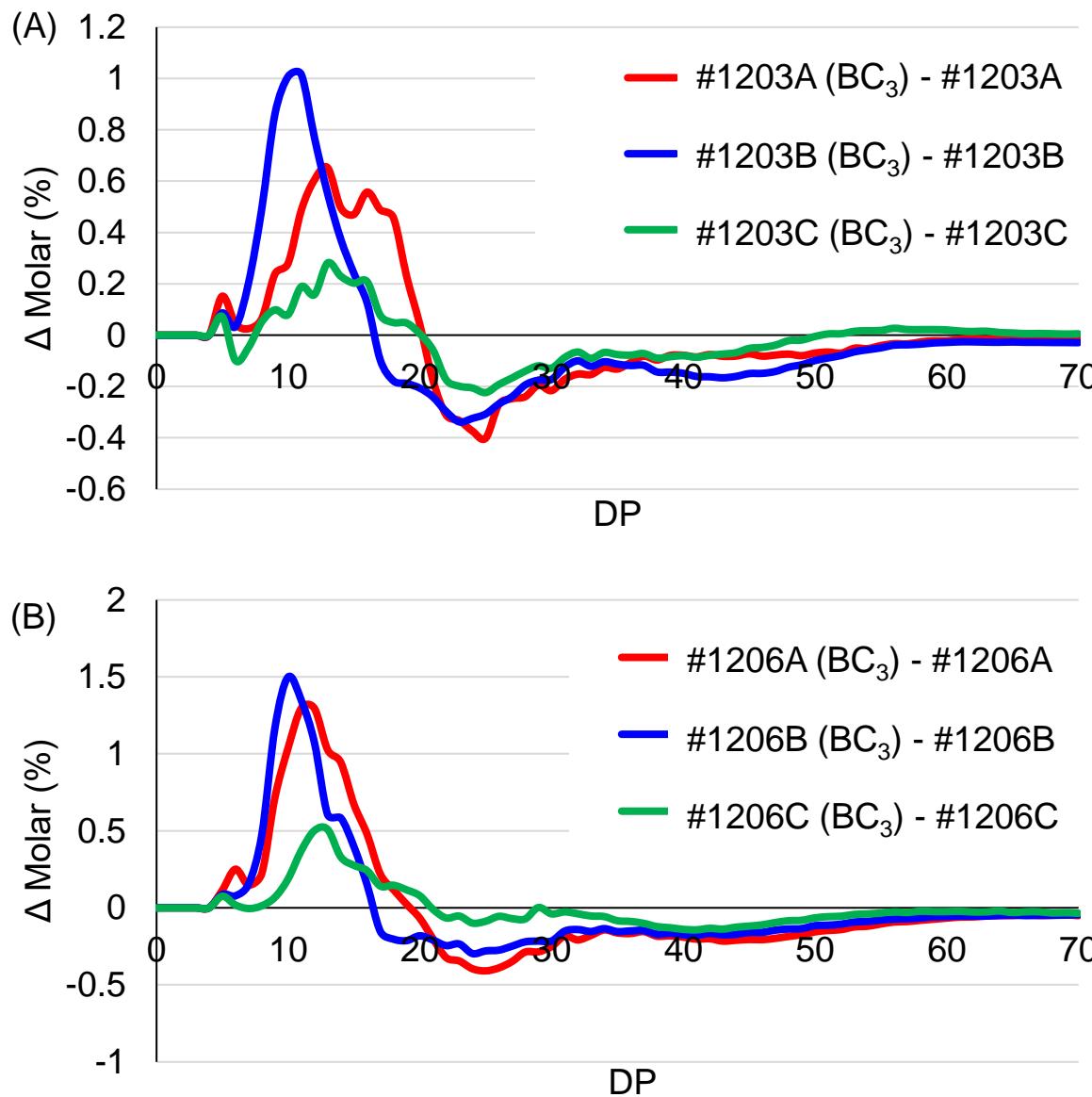
(B) After backcrossing



**Fig. S1B.** Pedigree of #1203 (BC<sub>3</sub>) and #1206 (BC<sub>3</sub>) lines after backcrossing.

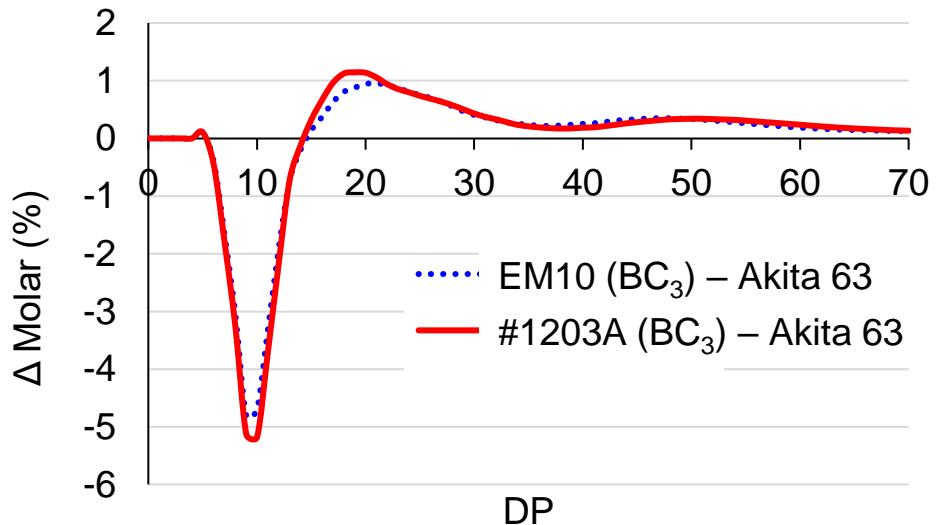


**Fig. S2.** Elution profiles of debranched endosperm starch separated by gel filtration chromatography. Fraction I contains amylose and extra-long chains of amylopectin; fraction II contains long chains of amylopectin; and fraction III contains short chains of amylopectin.

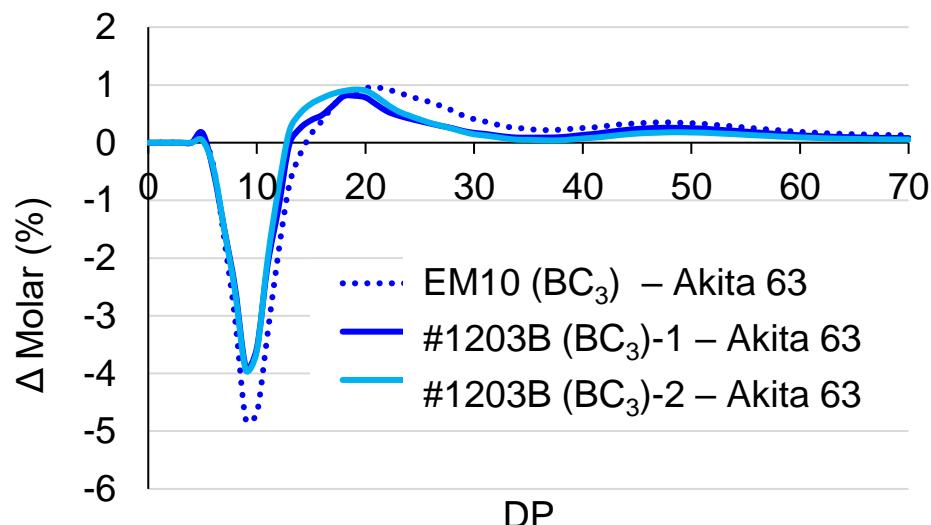


**Fig. S3.** Comparison of amylopectin structure before and after backcrossing. Differences are shown as  $\Delta$  Molar %, and the value was calculated by subtracting the pattern of before from after backcrossing, as indicated.

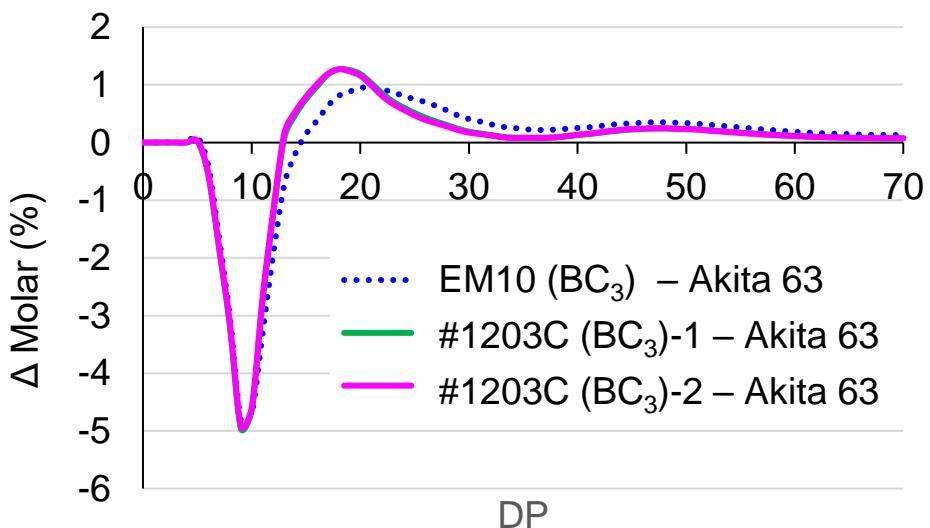
(A) #1203A: SS2a SS3a *gbss1<sup>L</sup>* *be2b*



(B) #1203B: ss2a<sup>L</sup> SS3a *GBSS1* *be2b*

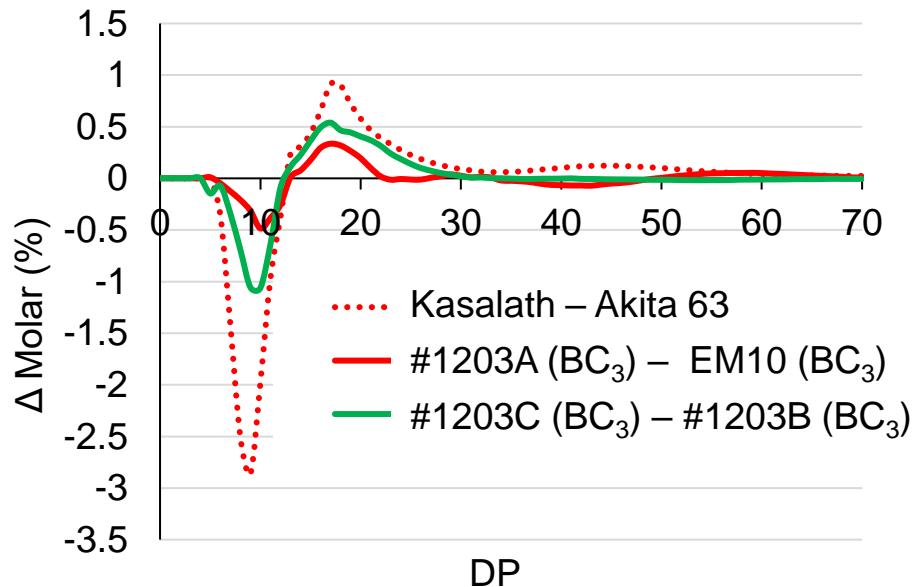


(C) #1203C: SS2a SS3a *GBSS1* *be2b*

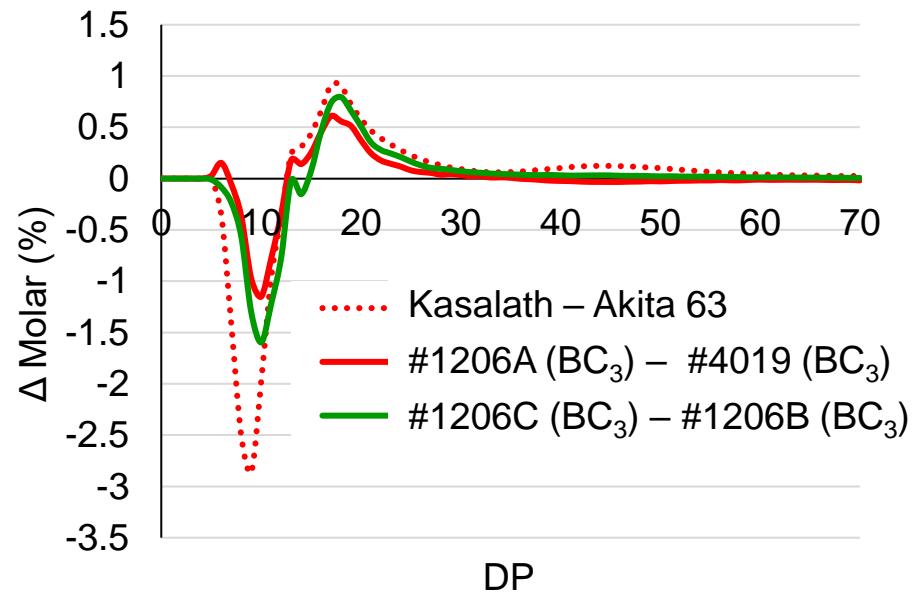


**Fig. S4.** Differences in amylopectin structure between #1203 ( $BC_3$ ) lines and Akita 63 to determine the effect of BEIIb loss in addition to the effects of SSIIa and/or GBSSI. Differences are shown as  $\Delta$  Molar %, and the value was calculated by subtracting the pattern of Akita 63 from each line, as indicated.

(A)



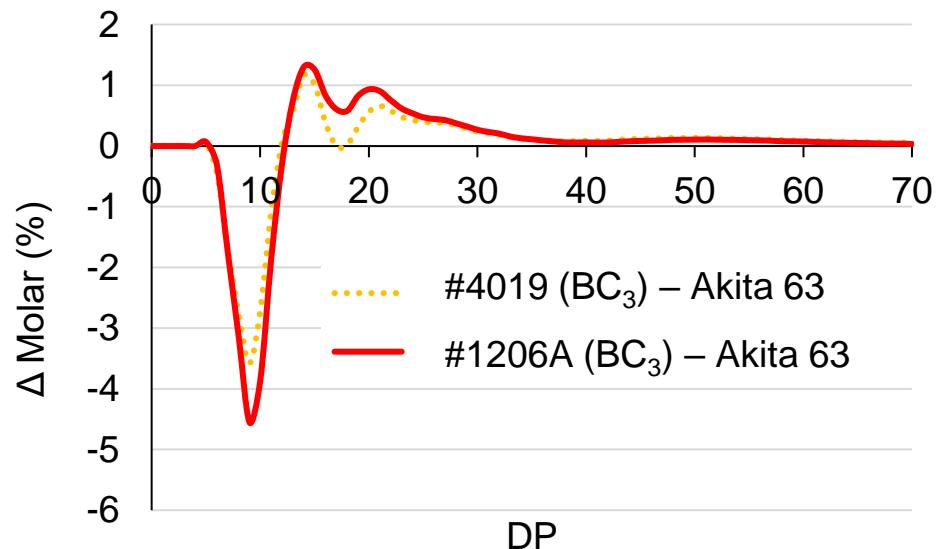
(B)



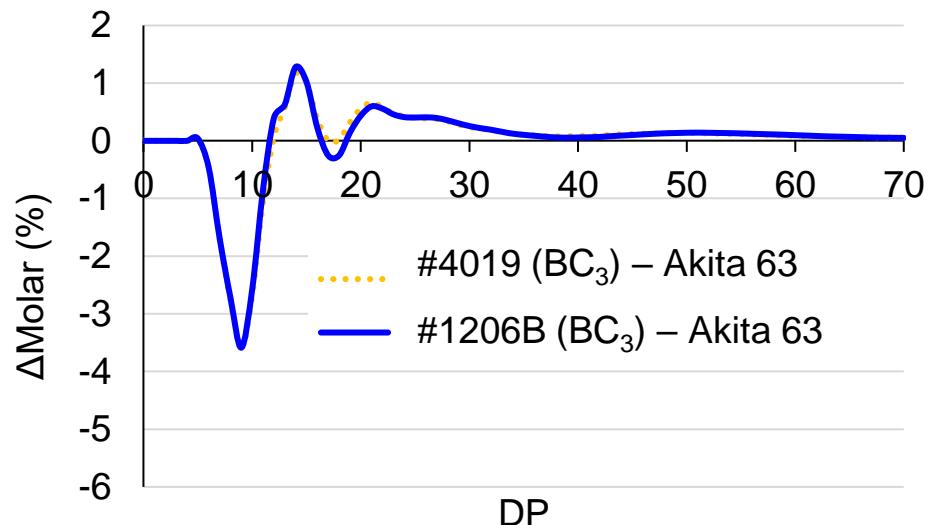
**Fig. S5.** Differences in amylopectin structure showing the effect of active SSIIa.

Differences are shown as  $\Delta$  Molar %, and the value was calculated by subtracting the pattern of less-active SSIIa ( $ss2a^L$ ) from active-SSIIa line (SS2a), as indicated.

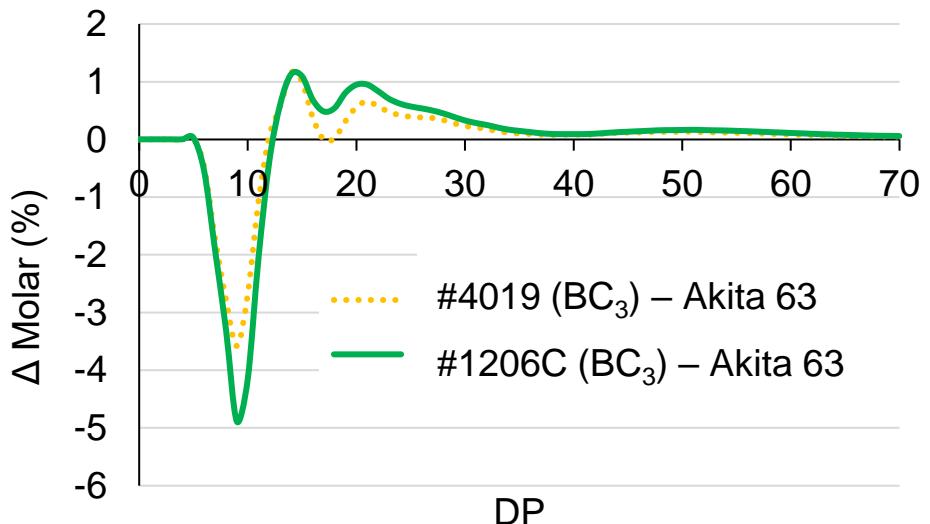
(A) #1206A ( $\text{BC}_3$ ): SS2a ss3a *gbss1<sup>L</sup>* *be2b*



(B) #1206B ( $\text{BC}_3$ ): ss2a<sup>L</sup> ss3a *GBSS1* *be2b*



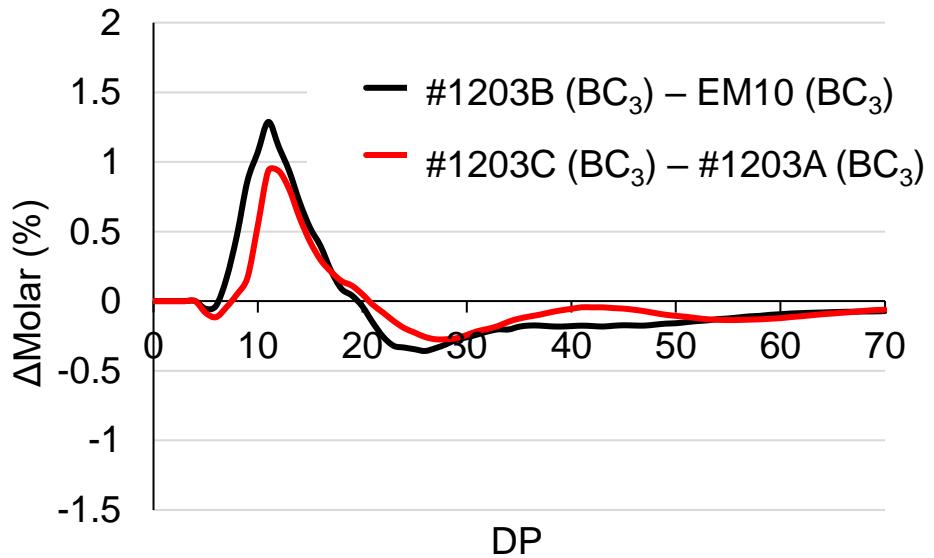
(C) #1206C ( $\text{BC}_3$ ): SS2a ss3a *GBSS1* *be2b*



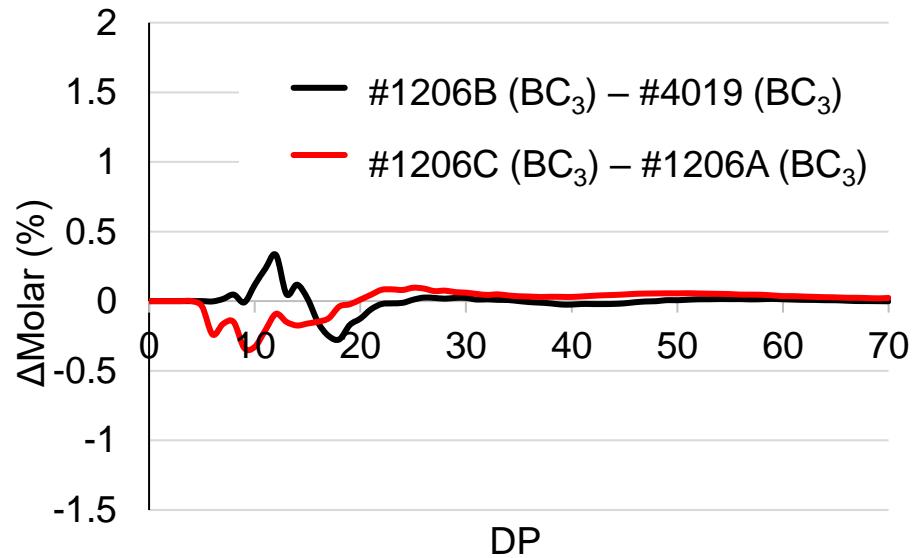
**Fig. S6.** Differences in amylopectin structure between #1206 ( $\text{BC}_3$ ) lines and Akita 63 to determine the effect of the loss of SSIIla and BEIIb in addition to the effects of SSIIa and/or GBSSI.

Differences are shown as  $\Delta$  Molar %, and the value was calculated by subtracting the pattern of WT from each mutant line, as indicated.

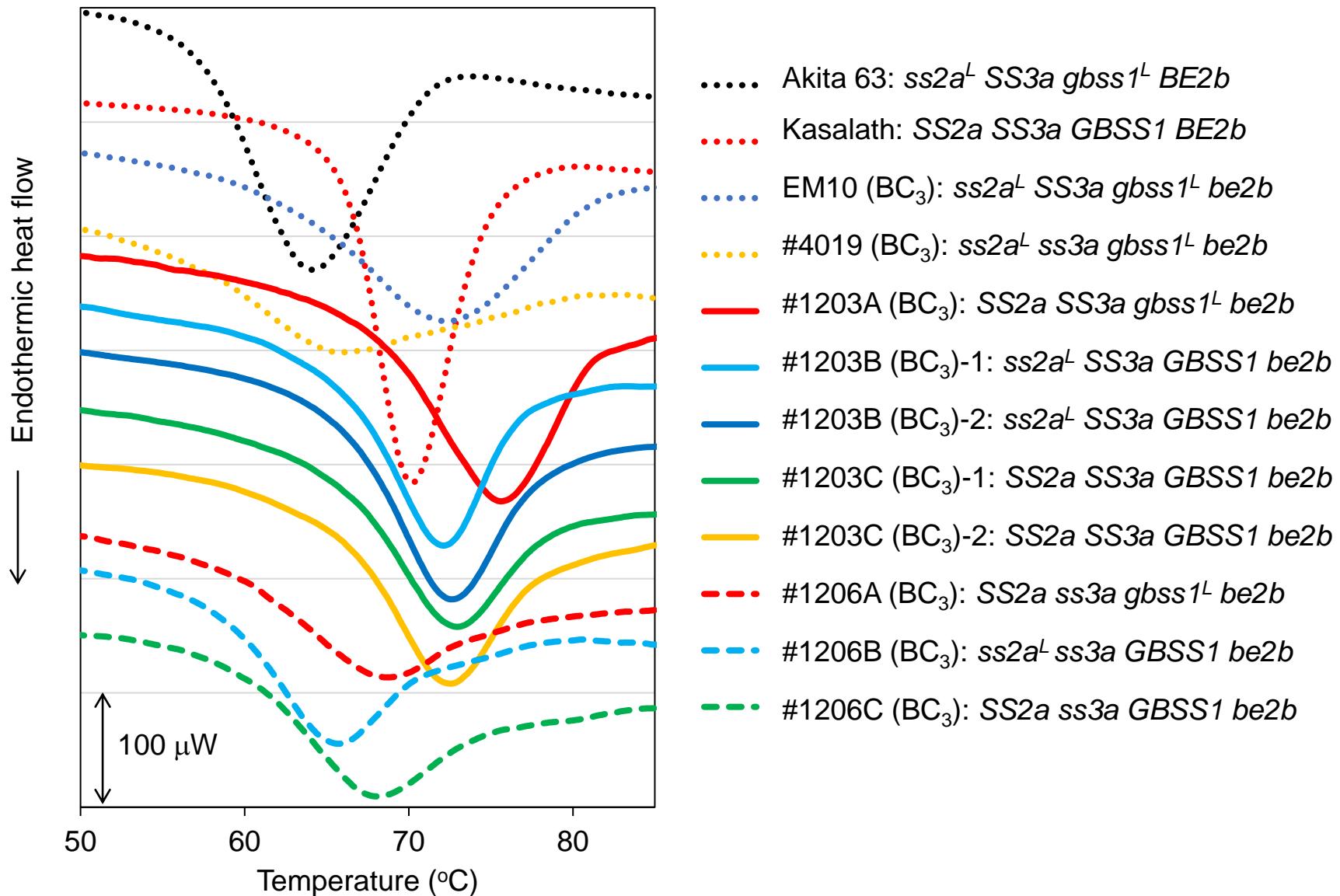
(A)



(B)



**Fig. S7.** Differences in amylopectin structure showing the effect of high expression level of GBSSI. Differences are shown as  $\Delta$  Molar %, and the value was calculated by subtracting the pattern of low-expression GBSSI ( $gbss1^L$ ) from high-expression GBSSI ( $GBSS1$ ) line, as indicated. Black line indicates a theoretical value calculated by adding the effects of the loss of BEI alone and BEIIb alone.



**Fig. S8.** Typical differential scanning calorimetry profiles of rice lines showing peak gelatinization temperature. Data represents one of at least three independent replications using purified starch.